Development of novel TGF-β-targeted therapeutics to treat cachexia

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Cachexia is a debilitating syndrome characterized by severe frailty and fatigue, associated with the loss of skeletal muscle mass. This condition presents in many diseases, including cancer, heart failure and AIDS, and the onset of cachexia greatly reduces quality of life and survival. Elevated levels of the transforming growth factor-β (TGF-β) signalling proteins activin and/or myostatin are reported in several conditions where patients experience wasting. In advanced cancer patients, up to 80% exhibit cachectic symptoms and, remarkably, 25% of cancer-related mortalities (1.9 million people globally in 2008) derive from cachexia rather than direct tumour burden (Tisdale, 2009). Activin and myostatin overexpression in mice correlates with varying degrees of cachexia (Zimmers *et al*., 2002; Chen *et al*., 2014). In order to combat excessive activin and/or myostatin signalling, we developed specific activin and myostatin antagonists by systematically modifying their prodomains and encoding them into recombinant adeno-associated viral vectors (AAV) as our delivery tool. To assess their capacity *in vivo*, we injected the AAVs directly into the *tibialis anterior* (TA) muscles of healthy C57Bl/6 and colon-26 (C26) tumour-bearing Balb/c mice. All mice were male between six to ten weeks of age and anaesthetized with isoflurane during AAV delivery and/or C26 tumour implantation. A single injection of AAVs encoding for the modified activin prodomain resulted in a 20% increase in TA mass after eight weeks, and the myostatin prodomain led to 40% hypertrophy. Remarkably, blocking both activins and myostatin, by combinatorial prodomain delivery, resulted in a muscle mass increase by 116%. Haematoxylin and eosin staining of cryosections demonstrated graded increases in muscle fibre size consistent with the increases in TA mass, and Western blot analysis revealed an increase in the phosphorylation of mTOR and S6RP, indicating activation of the protein synthesis pathway. Quantitative RT-PCR of activin/myostatin target genes *Mss51*, *Igfn1* and *Fbxo32* showed decreased expression with activin/myostatin inhibition. These data that suggest multiple TGF-β antagonism as a possible therapy for specific wasting conditions. Indeed, in C26 mice (where TA muscles waste 30%), AAV delivery of activin and myostatin prodomains completely prevented TA muscle loss, as the mass was indistinguishable from that of healthy mice. Current studies are evaluating systemic prodomain treatment to prevent/reverse muscle wasting in not only cachexia, but also in models of denervation, muscular dystrophy and sarcopenia.

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